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INFLUENZA: METHODS OF STUDY AND CONTROL[1]

The Wesley M. Carpenter Lecture[2]

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That influenza should be chosen as the subject for the Wesley M. Carpenter Lecture is a signal distinction and a recognition of the developments in the field during the past decade. In this time we move from the first identification in the Western Hemisphere of virus from epidemics of influenza in man, to the demonstration that prophylaxis against the epidemic disease is possible. In the advance there have been numerous contributors, many diversions and frequent discoveries of fundamental biological importance.

The probabilities for the control of an infectious disease increase as knowledge of its biology advances. There is no simple formula leading to a successful conclusion. Once the responsible agent is identified, however, information develops as tools and methods for promoting the study are devised. But in the study interest in the method should not

[1] From the Virus Laboratory and the Department of Epidemiology, School of Public Health, University of Michigan. Certain of these studies were conducted under the auspices of the Commission on Influenza, Board for the Investigation and Control of Influenza and other Epidemic Diseases in the Army, Preventive Medicine Service, Office of The Surgeon General, U. S. Army.

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obscure the problem of the disease: its identification, an understanding of the mechanisms by which it is produced, maintained, and distributed; an evaluation of procedures which might aid in the control or cure of the disease.

Since 1933, when Smith, Andrewes and Laidlaw,[1] following on the heels of Shope's[2] demonstration that a virus was involved in swine influenza, first identified a virus from human influenza, a large number of investigators in numerous laboratories in different countries have tackled the various aspects of the problem. While it is impossible to make a sharp division between studies of the virus and the application of this information to study of the natural disease it may be well first to review certain characteristics of the virus which serve as the basis for much of the wider research.

Studies of the Virus

Media: At present there is no direct method for the rapid identification of influenza virus in the clinical case. Its recognition depends upon the demonstration of effects produced in the course of its infectious activity. At first the response of the ferret was the sole indicator; then the mouse, squirrel, hamster and other species came into use. Later the embryonated hen's egg became important, with infection established by inoculation onto the choroallantoic membrane, into the amniotic sac, directly into the embryo, or simply of unfiltered throat washings into the allantoic sac. Beyond a characteristic febrile reaction in the ferret, demonstration of the virus' presence in animals, after intranasal inoculation of suspected material, comes from the development of a typical pulmonary lesion with continued passage or through the fact that the virus induces the production of antibodies in the blood and a state of resistance to reinfection. Originally the egg had somewhat the same general purpose as tissue cultures, for demonstration that virus was present depended upon secondary transfer to animals or serological procedures. In the important discovery first reported by Hirst,[3] later also by McClelland and Hare,[4] that influenza virus caused agglutination of erythrocytes of the embryonic or adult chicken an extremely adaptable reaction came into use. When a certain concentration of virus in the egg is reached it can be readily indicated by an immediate macroscopic effect. The possibility of rapid identification of influenza virus in the patient's secretions has been thereby enhanced so that in at least two instances the actual nature of an outbreak was established in about 4 days by inoculation of eggs.[5,6]

Antigenic Variation: Two types of virus are at present identified. Type A is the group of strains resembling the original WS strain of 1933. Strains of swine influenza also have antigenic relationship to Type A virus.[7,8] For a period it appeared on the basis of procedures employed at the time that the strains were antigenically identical but, together with Magill in 1937,[9] it was shown by more precise methods that even the strains exhibited detectable variations. As these studies expanded, more striking differences among them became apparent.[10] In the eggs the strain differences appear to be accentuated to such an extent that it represents one of the hazards of the method. On the other hand there is the possibility that the antigenic status as determined early in the egg is more truly representative of the human virus as it moves at large than that adapted to animals by repeated passages. Burnet has suggested the significance of still finer differences. He noted with one strain adapted to eggs by the amniotic sac that there was a greater capacity to agglutinate guinea pig cells than chick cells and that by selective adaptation, only agglutinins for guinea pig cells were developed.[11] Under other conditions the agglutinins for chicken cells developed and overgrew the cavean agglutinins. He suggested that the guinea pig agglutinins were those of the original human strain -O- while agglutinins of chicken cells were the result of a derivative -D- developing in the egg—thus a variant of the original. These interpretations do not appear to be supported in the observations of Rickard and his associates or those of our laboratory where virus has been identified after its first inoculation into eggs, by the agglutination of chicken cells to a high titer without significant differences in its effect on guinea pig cells.[12] Although certain of the variations detectable by increasing refinements in technique seem to be of academic interest, evidence continues to accumulate indicating that they are of practical importance as well.

Type B influenza virus was discovered in 1940 but evidence showed that it had been prevalent in the epidemic of early 1936.[13,14] Its general behavior in animals and eggs is essentially the same as that of Type A. It has, however, been consistently milder in its pathogenicity for animals and relatively few strains of the virus have been isolated. But despite the otherwise close similarities, immunologically Type B virus is so distinct from Type A as to represent an entirely different agent. The difference is much greater than the variations between strains of Type A so that hyperimmune serum which largely obliterates strain specificity and overlaps the boundaries between Type A and swine viruses fails to show cross reactions between A and B.[15]

The importance of these sharply distinguished entities to an understanding of the disease problem will be clearly seen later in the discussion.

Serological Reactions: In response to exposure to influenza virus, whether by infection or otherwise, antibodies which may be measured in a variety of ways can be demonstrated in the blood stream. The capacity of the virus to infect animals can be neutralized by mixing it with serum of the recovered animal; the neutralizing or protective antibodies are most commonly measured in mice by determining how little serum added to a known amount of virus is required to prevent infection when the mixture is given intranasally. In this manner it was first shown that a large percentage of human individuals after the first few years of life, presumably as a result of natural infection, had antibodies to influenza A[16, 17] and that despite this fact they might well acquire the disease again with a further rise in titer. The response was so clearly correlated with infection by Type A influenza virus and not with other infections that its specificity was established.[18] Infection with Type B virus has no influence on the titer to A, and vice versa.[13, 14] And now the demonstration of an increase in titer between serum taken in the acute phase of illness and that in convalescence is generally diagnostic.

Recently, Hirst's observation[19] that antibodies to influenza virus would prevent the virus from agglutinating chicken erythrocytes has resulted in still more simplified serological procedures, which can be carried out rapidly in a general laboratory.

It is interesting in this respect that here again there is a sharp specificity between Type A and Type B viruses in that the respective immune sera inhibit only the homologous virus from causing agglutination.

It is not desirable in the scope of the present discussion to dwell too long on these measures of the virus activity although they represent largely the underlying methods upon which much of the subsequent information is based. There are other lines of physico-chemical research which have been increasingly attractive. The size of the virus has been measured under a variety of conditions and except for one brief excursion into much smaller realms its diameter is at present still somewhere between 70 to 100 Mu.[20,21,22,23] Efforts to characterize the virus by electron microscopy have yielded photographs of rounded bodies with relatively high homogenicity which appear to possess the virus activity.[22,24,25] The active component freed from much extraneous material has also been obtained in sufficient amounts to foster attempts to identify the chemical composition of the influenza viruses. But at the risk of appearing to minimize the importance of these splendid studies of fundamental biological significance, I should like to turn to the body of investigations which are more concerned with the clinical and epidemiological aspects of the disease.

Pathogenesis

An understanding of the mode of action of influenza virus is important since it serves to orient many activities which might appear at first glance unrelated. Investigation shows that influenza viruses act quite specifically upon the respiratory tract. Except that certain strains have been shown to be adaptable to the nervous tissue in mice[26,27] and that an hemorrhagic encephalitis, similar to that produced by many viruses, is observed in infected chick embryos,[28] influenza virus in swine, ferrets, mice, chick embryos and man exerts its pathogenic effect on the epithelium of the respiratory system. When virus is administered in relatively large amounts by pararespiratory routes, the disease is not produced whereas a minute amount intranasally sets up the infection. The injury is largely a destruction of the typical ciliated columnar epithelium which lines the larger respiratory divisions and which the virus reaches ordinarily by entry into the lumen. This is the essential tissue to which protection must be furnished in order to prevent disease. When moderate amounts of virus are given intraperitoneally or intravenously to mice, virus can be recovered from the lungs but pulmonary lesions do not develop. This is probably accounted for by the fact that the virus is on the wrong side of the susceptible cell and only when it floods over into the upper respiratory tract will it gain access to the epithelial lining. The pneumonia which develops in experimental animals, while related to the virus injury, is apparently a lesion secondary to epithelial destruction and the subsequent serous exudation.

The effect of bacteria upon the pathologic process in man is not well known since influenza of recent years has not been associated with a high incidence of complications. In several instances, however, observers have discovered simultaneous prevalence of influenza A or B and hemolytic streptococcal infection without too great evidence of symbiotic effect.[29] Shope has clearly shown, however, the exaggeration of injury produced when swine influenza virus and H. influenzae suis are given together.[30] A mild illness is converted into a serious disease. As yet unpublished studies from our laboratory have demonstrated in mice that strains of H. influenzae, which by themselves are harmless intranasally, when given several days after a minimal non-fatal virus infection is induced, can establish themselves, multiply and bring about a lethal result. Moreover, it appears that in some instances the virulence of the bacterial strain is enhanced.

It is apparent, however, that the virus is the major component in the origin of influenza and the disease which has been studied in man has been largely an uncomplicated infection. Consequently, much of the discussion deals with information gained under these conditions in relation to specific cellular injury by the virus.

The Clinical Disease

During the course of an epidemic of influenza there is a surprising degree of uniformity in the clinical picture presented. Nevertheless, studies of the virus infection reveal, as with most diseases, that severity varies from the unrecognized subclinical invasion in a relatively large proportion of the population to the severe fatal disease in which pulmonary involvement is prominent. But that they represent infection by the same virus has been amply demonstrated by the recovery of the virus and by a study of the serological responses.

On the basis of studies in the past decade influenza A tends to elicit a sharp clinical disease with abrupt onset, fever and pronounced constitutional symptoms of three to four days' duration. Even in the milder cases the course tends to follow the same pattern. On the other hand several reports have noted that in comparison influenza B has had a more gradual onset,[31, 32, 33, 34] the disease was less intense and the duration of the fever shorter. Several of the writers describe the onset as a common cold and some observers have noted in children, especially, almost an absence of significant complaints. These impressions are substantiated to some extent by our experience with a group under close observation. Influenza B occurred in nearly 25 per cent of the population in a large institution as demonstrated by serological means although only 4 clinical cases were recognized.[35] However, the epidemics of influenza B which were studied in 1936 and 1940 revealed a large body of cases with clinical illness quite typical of epidemic influenza.[13, 31] Nigg and her associates reported 4 cases of fatal pneumonia during a limited outbreak of influenza B.[36] In a prevalence a year ago among a group of 100 elderly women, three fatalities occurred out of thirty-one cases. One point noted in the 1936 and subsequent epidemics of influenza B which differed somewhat from that usually seen in influenza A is the not uncommon tendency to nausea and vomiting.

Further evidence of the clinical features has been gained from infection experimentally induced in human subjects by inhalation of virus.[37-41] It is quite striking that while signs of parenchymal involvement have been noted in a proportion of cases, pneumonia has been a rare complication. The incubation period with experimental influenza A has been 24 to 48 hours and the onset abrupt. Chills or chilliness, fever, cough, headache, general aches and prostration of two to three days' duration have been the rule. Nasal discharge has been less frequent. A considerable degree of lassitude follows the decline of fever. With experimental influenza B the incubation period has in general been shorter, not uncommonly twelve to eighteen hours. This brief incubation period is a striking observation and that it represents the effect of active virus is seen by the fact that irradiated material does not produce clinical disturbance. In addition, the course of experimental influenza B has been milder than that of influenza A; fever has usually been of no more than one day's duration and recovery appears to be more rapid. Even under these circumstances nausea and vomiting has been not infrequent.

The illnesses produced by these two viruses offer certain contrasts with the form of upper respiratory infection most commonly associated with atypical pneumonia. It is quite certainly *not* influenza A or B. The onset is usually much more gradual with symptoms of respiratory irritation appearing early. Nasal congestion, cough, hoarseness, substernal soreness tend to develop progressively but the patient does not usually present the same degree of prostration. The leukocyte count is not so uniformly low. While these differences appear to be of minor nature, they tend to be quite prominent when seen in large groups.

In the individual patient, however, because of the wide variations that may be encountered the difficulty in diagnosis is increased. And since many of the symptoms can be found related to the onset of a great number of diseases, there is still no simple test which permits a prompt diagnosis at a single glance. Efforts which seek to find specific clinical methods for identifying the various etiological entities have revealed the probability that still other types of influenza virus will be discovered, and that unrelated viruses will be found in some of the respiratory infections which at present prevail. The importance of gaining this knowledge cannot be overemphasized since it constitutes the information through which specific preventive or curative measures can be devised.

Epidemiology

Just as identification of influenza virus infection serves to chart the clinical boundaries so it is a valuable procedure in giving information as to the natural history of the diseases, for in this manner the wanderings of the virus can be detected even when they are not suspected through clinical or epidemiological observation. For several years, together with other investigators, it was my opinion that influenza A was almost exclusively an epidemic disease. This was based to a large extent upon the fact that the periods in which the virus had been shown to be present were occupied by epidemics. Moreover, for nearly nine months in 1940 a constant sampling of respiratory infections in the wards of the Third Medical Division, Bellevue Hospital, was made by I. J. Brightman.[42] The first case of influenza A was identified at the end of this period in December when an epidemic began in the city. There was thus little evidence of scattered sporadic cases.

From the winter of 1932-33 when the virus was first isolated until 1940-41, five outbreaks in alternate years had been identified. The cycle skipped a beat in 1942-43 and the epidemic appeared last winter after a three-year interval. But despite the fact that the usual epidemic did not occur during the winter of 1942-43, influenza A was found to be in operation. In Canada, Hare and others observed a sudden, self-limiting, brief flurry in an Army group in April 1943;[33, 43] in England, spotty group infections unrelated to any general incidence were detected during that summer.[44] In Australia scattered cases were detected in the same months.[34] In our studies serological evidence was obtained of three possible cases in February and March while in May, 3 definite cases, from one of which virus was recovered, suddenly appeared at an army post without any evidence before or after of an epidemic prevalence.[35] It was not until early November that epidemic disease became obvious. These observations covering wide geographic areas at the same time intervals clearly show that influenza does appear in sporadic fashion and it is not an unlikely probability that these episodes represented the stones from which the 1943-44 epidemic was built.

It has been observed that epidemics vary quite definitely in their scope and intensity. For instance, the outbreak of 1936-37, occurring the world over, was moderately severe and distributed generally throughout the population; while that of 1938 to 1939 represented a mild form recognized largely in institutional groups. The same characteristics were observed in other countries that year. Hence, influenza A has been shown to change the pattern of its infection widely: pandemic, epidemic, endemic or sporadic. It is extremely interesting to point out, however, that the form it takes at a given time tends to typify its behavior at widely distant points.

The smouldering scattered distribution recently detected with influenza A, seems to be the more common experience with influenza B. In most accounts of the past three years there has been reference to influenza B scattering through the population without definite indication of an epidemic unless it be in a limited group.[32, 33, 34, 35, 44] It has been repeatedly mentioned that many cases of unidentified illness occurred at the same time as the cases of influenza B. Observations of the distribution of antibodies to influenza B in the population are in accord with this. One series, as yet unpublished, found much lower titers against B than with A but an occasional individual exhibited a high titer suggesting recent infection. Nevertheless, influenza B can cause widespread marked epidemics such as were observed in 1936 and 1940. Hence, the pattern in influenza B is also inconstant.

The above observations tend to indicate that the influenza viruses are in constant circulation. Evidence for human carriers of the viruses is, however, not present. Shope has suggested that swine influenza virus represents the 1918 human strain[30] and he has observed swine infection with more recent strains of Type A virus.[45] Can swine serve as a reservoir for the human disease? Shope has also presented evidence that swine influenza virus is maintained in a masked form in the lung worms parasitizing swine from which it erupts when provoking influences disturb the equilibrium.[46] This would account for the storage of virus between epidemics and the explosive manner in which many of them arise. The possibility that some similar mechanism serves to preserve the agent in the human body has not been explored. Nevertheless, there is ample evidence that influenza can be transmitted from man to man and at the moment this seems to fit best the facts concerning the spread of the disease.

Efforts Toward Prevention

The foregoing remarks immediately illustrate certain of the problems which enter into prevention of influenza. But it may limit some of the obscurities if we take as a starting point the thesis that the essential requirement of preventive measures is to prevent the virus from reaching the susceptible respiratory epithelium in such a form as to inflict its characteristic injury. Studies over several years have shown that the respiratory secretions contain antibodies to influenza virus, apparently derived from the blood, which may represent the most efficient first line of defense since when present they are readily available to the tissues which they bathe.[47, 48] They are not constantly present, however, nor are they present in large amounts. Nevertheless, it seems that procedures which augment this mechanism may well be effective in procuring protection from the disease. It has been found that in recovery from natural infection the protective capacity of the nasal secretions is enhanced but there is as yet inadequate information as to how long immunity persists. Indications are that it is much longer than is ordinarily said to be the case, probably for a period of years against the same virus. Nevertheless, if infection be induced by a virus so attenuated as to avoid clinical injury it might be applied as needed and build up, through subclinical infection, a relatively durable resistance. Certain reports by Bull and Burnet[49] have yielded results which they considered suggestive but in our studies under a variety of conditions the response to avirulent strains has been too irregular for widespread testing.[50] In one investigation individuals who had developed clinical disease after inhalation of influenza virus, Type B, were subjected to the same procedure with the same virus 4 months later.[39] About one-third again reacted with well marked clinical disease, clearly indicating that a solid immunity had not persisted for this interval against the amounts of virus employed in the test. The severity of the test is emphasized by the fact that nineteen of twenty-three control individuals came down at the same time. The possibilities along this line have certainly not been exhausted and there are still many reasons for further investigations of immunization by inhalation.

The most widely studied method for attempting to increase immunity has been that of subcutaneous vaccination. It was stated earlier that subcutaneous inoculation of active virus does not produce infection. In animals it can be shown, however, to result in the production of protective antibodies and of resistance. It has been shown also that with proper subcutaneous vaccination the virus-inactivating capacity of the nasal secretions is also enhanced.[51] A number of studies using preparations of virus derived from mouse lung, tissue culture, chick embryo or allantoic fluid have been tested.[38-41, 52-65] In many instances no disease arose to test the result; in others suggestive results have been obtained.

In the winter of 1942-43 under the auspices of the Commission on Influenza, Army Epidemiological Board, 8,000 people in two institutions were included in a vaccination study which employed inactive virus concentrated from allantoic fluid. Studies of the antibody titers before and after vaccination in 419 of the 4,000 vaccinated individuals revealed that over 90 per cent showed a sharp increase in two weeks.[64] Studies in a smaller group, over a longer period of time, indicated that a slight decline had occurred after 4 months, and at the end of one year the distribution of antibody titers remained well above that before vaccination.[6]

No recognizable epidemic occurred during the winter immediately following vaccination. In an effort to gain information as to what benefit had been attained two groups of approximately 100 individuals each were tested for resistance to infection by intranasal inhalation with Type A and Type B viruses, respectively.

The results indicated that vaccination two to four weeks before infection had a pronounced effect. The influence of vaccination against influenza B[41] appeared to persist during a 4 months' interval while against influenza A this was less evident.[40]

During the epidemic of influenza A which occurred in the winter of 1943-44 the distribution of disease in this population might be construed to indicate a persistence of benefit gained from vaccination a year earlier.

On the basis of the experimental studies just detailed an extensive program for the winter of 1943-44 was planned and carried through by members of the Commission on Influenza in an effort to evaluate the effectiveness of vaccination against the natural disease. Results of previous field trials by other investigators suggested that vaccination had a beneficial effect, although the degree of reduction in incidence of disease in vaccinated as compared with control individuals was not sufficiently great to warrant, without further study, the use of this procedure on a wide scale.

The study of last winter, representing the coördinated efforts of members of the Influenza Commission and their associates in six laboratories in different parts of the country, was conducted in A.S.T.P. units at eight colleges and universities and in five New York medical and dental schools. Approximately 12,500 men were involved.[29]

In New York City two studies were organized, one from the laboratories of the International Health Division of the Rockefeller Foundation, by George K. Hirst, Major Norman Plummer and William Friedewald working in the A.S.T.P. units at the College of the City of New York, Princeton University and Rutgers University; the other, originating from the laboratories of Cornell Medical College, was carried out at the five New York medical and dental colleges and at Cornell University in Ithaca by Major Norman Plummer, Thomas P. Magill and Wilson G. Smillie. Rickard and his associates carried out a study at the University of Minnesota; Hale at the University of Iowa; Eaton and Meiklejohn at the University of California and a similar program was conducted from our laboratory at the University of Michigan.

The same preparation of vaccine was used by all investigators. Virus for the vaccine was grown in chick embryos and harvested and concentrated from the extra-embryonic fluids. The viruses of influenza A and B were included in the vaccine and were rendered non-infectious by the addition of formalin. Alternate men in each company were given a single, subcutaneous injection of 1 cc. of either the virus vaccine or control material.

In seven of the nine units, the interval between completion of vaccination and onset of the epidemic of influenza A varied from seven to thirty-one days. In two units, City College of New York and the University of Iowa, vaccination was begun after onset of the outbreak.

The incidence of clinical influenza in the vaccinated group comprising 6,263 men was 2.2 per cent and in the control group of 6,211 the incidence was 7.1 per cent. In almost all localities the trend was the same. Marked deviation from the average result was observed in only one unit where little difference between vaccinated and controls was evident. In the majority of units three to four times as many cases occurred in the controls as in the vaccinated groups. In two units, ratios of five and six to one were recorded. The factors responsible for these variations are not yet understood but may become apparent after analyses of the results are completed.

From the combined results of the study in all units, it would appear that vaccination was effective in reducing the incidence of influenza to about one-fourth, assuming that the incidence of 7.1 per cent observed in the control group was the expected incidence in a normal population. That this assumption may not be justifiable is suggested by observations made in totally unvaccinated companies at the University of Michigan where the incidence of influenza was about 20 per cent to 30 per cent as opposed to 8 per cent to 9 per cent in the control half of the vaccination study groups. If these differences are significant, it suggests that vaccination was of benefit to a proportion of the controls by virtue of their dilution with an equal number of vaccinated men with whom they were in constant association. The effect of vaccination in reducing the incidence of influenza may be greater than is indicated by comparing vaccinated and control subjects in a single homogeneous population. This may be another example of a fundamental epidemiological principle that has found application in the control of diphtheria and other epidemic diseases.

At City College of New York and the University of Iowa, where vaccination was begun while the epidemic was in progress, the morbidity rates were the same in treated and untreated groups, until the end of the first week after vaccination. Thereafter the difference became evident.

At the University of Michigan, during the prevalence of influenza A, an attempt was made to determine the effect of vaccination upon the incidence of the milder forms of respiratory illnesses unaccompanied by fever. All cases with symptoms of respiratory disease reporting to sick call were studied. Those with temperatures of 100° or more were hospitalized while those who exhibited no significant febrile reactions were seen almost daily in the dispensary until symptoms subsided. The great majority of hospitalized cases was diagnosed as influenza. That a high percentage of these was due to the virus of influenza A was confirmed serologically in about 90 per cent of the unvaccinated group. The afebrile illnesses studied in the dispensary were classified as influenza, local respiratory infection, or cold, depending upon the clinical impression. Except for the absence of significant fever, patients with influenza seen in the dispensary presented essentially the same syndrome as did the hospitalized cases. That the virus of influenza A was etiologically related to the illnesses observed in the majority was demonstrated by appropriate laboratory tests. In the group of cases diagnosed as "local respiratory infections," symptoms were confined to the respiratory tract and there were signs suggesting localized infection, such as sinusitis, pharyngitis, etc. The colds consisted of cases in which the presenting and predominant symptoms were those of rhinitis.

A division between vaccinated and controls within each diagnostic category reveals interesting variations. Of the hospitalized influenzas, the greater proportion were contributed by unvaccinated persons, while of the milder cases, such as ordinarily would not come to the attention of physicians in civilian practice, the differential was less marked. Thus, while the incidence of influenza of all degrees of severity was less in the vaccinated half of the population, in a significant proportion of those in whom infection did occur vaccination appears to have reduced the severity of illness.

The fact that no significant difference is apparent in the incidence of cases diagnosed as local respiratory infections and colds in vaccinated and controls, suggests that the vaccine had a rather specific effect. However, serological study indicates that, during the height of the outbreak, a number of these cases probably were mild infections caused by the influenza virus. It would appear that control and vaccinated individuals contributed equally to the incidence of respiratory illness with the mildest manifestations.

A point of interest noted in all study groups was that the difference in incidence of influenza in treated and control groups was greatest during the height of the outbreak, and as the epidemic subsided the difference between the two became less marked.

Results of this set of studies represent for the first time a clearcut demonstration that vaccination with inactivated virus by the subcutaneous route is capable of protecting to a significant degree against natural epidemics of influenza. They do not indicate, however, that the solution is complete. There is still need for the evaluation of the most effective strains to be employed so as to give the widest range of immunity; there is the question of the optimal amount of virus which can be used; beyond a certain point the possibility of toxic reactions enters; how long does the immunity last and can it be bolstered by multiple rather than single doses; what methods of production of material or even of immunization are to be the most practicable? But above all the problems of technique, it must be kept in mind that it is the production of immunity, not the production of virus, with which we are ultimately concerned. It is also important to bear in mind that the presence of antibodies and immunity are not synonymous, especially in the human individual where a ceiling on antibodies seems to exist.

Passive Immunity: The prophylactic approaches which have been discussed are those which tend to induce active immunity by modified infection with active virus or vaccination with inactive material. In both these instances it has been suggested that the effect obtained may reside largely in the influence upon secretions of the respiratory tract. If the superficial cells lining the air passages represent the vulnerable tissues, might it not be possible to protect them by applying immune substances directly at the surface? In other words, the action of the secretions might be augmented by antibodies introduced directly by the respiratory route. Smorodintsev and his associates[66] were the first to report efforts to study this possibility in the human subject. They subsequently stated that by spraying relatively small amounts of serum which were inhaled by the exposed subject, a marked reduction in the incidence of the disease during an epidemic was obtained.

Studies in experimental animals, largely mice, have repeatedly indicated that serum given intranasally has far greater efficacy than when given by other routes. In spraying for prophylactic purposes, however, it should be noted that excessive amounts of serum over long periods of time have been required to permit any significant results to be observed. On this basis alone one might have certain reservations as to the applicability to the problem of human influenza. In an effort to gain an impression of its usefulness, studies among human volunteers were carried out by members of the Influenza Commission. Serum was tested for its capacity to prevent infection by virus sprayed into the nostrils. Details of these studies have not yet been reported but it can be mentioned that substantiation of the statement of the Russian workers was not indicated.

This lecture has touched rather broadly on a wide variety of methods of study which have been employed during the past ten years in the investigations of influenza. It has been seen that knowledge of the viruses, the diseases they produce, and their distribution in nature, have been approached directly. It has become increasingly apparent as well that in order to determine the value of procedures which might serve in the prevention and control of the human disease information must be obtained in human subjects. In this respect somewhat venturesome utilization of the human volunteer has been most profitable, since it permits evaluation of the different proposals in the host concerned. Whether one can consider the experimentally induced infection strictly comparable to the natural disease is not a question, but it does furnish a procedure for investigation. At the moment, the procedure which has given the most definite evidence of limiting susceptibility to influenza has been subcutaneous vaccination. We have indicated, however, that much remains to be done.

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